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A new genus and species of cave-dwelling leech from China in the family Salifidae: taxonomy, phylogeny, and mitochondrial genome characterization

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Abstract

Accurate identification of leech species is critical for biodiversity conservation and evolutionary studies. However, due to the ambiguity and variability of some key characteristics, Salifidae remains a rather poorly known group of Erpobdelliformes. In this study, a new genus of salifid leech, *Troglobdella* **gen. nov.**, is established, and a new species, *Troglobdella guizhouensis* **sp. nov.**, is described based on specimens collected from a cave in Guizhou Province, Southwest China. Phylogenetic analyses based on mitochondrial cytochrome c oxidase subunit I (COI) and nuclear 18S rRNA gene sequences reveal that *Troglobdella guizhouensis* represents a distinct lineage within a clade that also includes the troglobiotic species *Shibabdella wulingensis* Tang & Liu, 2025, and the terrestrial *Odontobdella gaowangjiensis* Yin & Liu, 2025, both of which are known from Hunan Province. This suggests that cave-related morphologies in Chinese salifid leeches may have evolved independently. The complete mitochondrial genome of the new species was assembled into a circular molecule of 15,732 bp, comprising 13 protein-coding genes, 22 tRNAs, and 2 rRNAs. This study contributes new insights into the taxonomy, systematics, and mitochondrial architecture of cave-adapted leeches.

Key Words

mitochondrial genome, phylogeny, Salifidae, taxonomy, *Troglobdella guizhouensis*, troglobiont

Introduction

Leeches (Annelida: Hirudinea) are a diverse and ecologically significant group of segmented worms characterized by a dorsoventrally flattened body and the presence of anterior and posterior suckers. Most species are carnivorous or hematophagous, playing important roles as predators, parasites, or scavengers in aquatic and terrestrial ecosystems. Globally, more than 700 leech species have been described, reflecting their wide biogeographic range and considerable variation in ecological niches and life-history strategies (Phillips et al. 2020; Tong et al. 2022).

The family Salifidae, within the order Arhynchobdellida and suborder Erpobdelliformes, includes leeches that primarily inhabit freshwater environments, with some species adapted to semi-terrestrial habitats. These leeches are characterized by a microphagous feeding strategy, typically preying on small aquatic invertebrates such as annelids, mollusks, and arthropods. Morphologically, they are characterized by a strepsilaematous

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pharynx often equipped with myognaths bearing stylets (Yang 1996). Despite these traits, the taxonomic resolution within Salifidae remains challenging due to morphological homoplasy and variability in key diagnostic features, making the group one of the least understood within Erpobdelliformes.

Historically, eight genera have been recognized within Salifidae: (1) *Salifa* Blanchard, 1897; (2) *Barbronia* Johansson, 1918; (3) *Dineta* Goddard, 1909; (4) *Linta* Westergren & Siddall, 2004; (5) *Mimobdella* Blanchard, 1897; (6) *Odontobdella* Oka, 1923; (7) *Scaptobdella* Blanchard, 1897; and (8) *Sinodontobdella* Nesemann et al., 2007 (Nakano and Nguyen 2015). These genera include species that occupy aquatic habitats or moist terrestrial microhabitats. More recently, a troglobiotic genus, *Shibabdella*, was described from a karst cave in Hunan Province, representing a rare example of cave-related traits within the family (Tang et al. 2025). The discovery of *Shibabdella* suggests that Salifidae may harbor unrecognized diversity in extreme or understudied environments, such as subterranean ecosystems.

Karst caves in the Yunnan-Guizhou Plateau of Southwest China represent highly specialized and isolated habitats that host a variety of troglobionts, organisms unable to maintain viable populations outside the subterranean realm. These cave ecosystems are ecologically distinct from surface habitats due to their stable microclimate, lack of light, and limited nutrient inputs. Cave-dwelling animals often exhibit convergent morphological traits, such as reduced pigmentation, loss of eyes, and elongation of sensory structures (Liu 2021). Although cave leeches are globally rare, they have been sporadically recorded in various regions, including Erpobdellidae in Western Asia and Europe (Kerovec et al. 1999; Cichocka et al. 2015; Sket et al. 2021), and Haemadipsidae and Salifidae in China (Yang et al. 2009; Huang et al. 2019; Tang et al. 2025).

In the present study, we establish a new genus of cave-dwelling leech and describe a new species within it based on specimens collected from a karst cave in Guizhou Province, Southwest China. We present a comprehensive morphological diagnosis, phylogenetic analyses based on mitochondrial COI and nuclear 18S rRNA gene sequences, and a complete mitochondrial genome. These results clarify the systematic position of the new taxon and contribute to a better understanding of the evolutionary diversification of cave-dwelling Salifidae in East Asia.

Materials and methods

Specimen sampling and morphological observation

On January 19, 2024, a total of 14 specimens were collected from several shallow puddles located inside Chishui Cave (GPS coordinates: 26°53'42"N, 104°19'59"E; altitude: 2334 m), situated in the Yangjiao Group, Zhaoshan Village, Shanqiao Street, Caohai Town, Weining County, Guizhou Province, China. The specimens were transported alive to the laboratory, where they were relaxed in 15% ethanol and then fixed in 95% ethanol for preservation. Specimens were used for photography, morphological measurements, dissection, and molecular analyses.

Six type specimens (one holotype and five paratypes) were measured using a digital caliper with a precision of 0.1 mm. Four specimens were selected for anatomical study. The reproductive system was examined by dorsally dissecting the specimens and pinning them onto a wax tray. Morphological characteristics of the holotype were documented and photographed using a stereomicroscope equipped with a digital imaging system.

DNA extraction, PCR, and DNA sequencing

Genomic DNA was extracted from the caudal suckers of two specimens, which were excised with a scalpel, immediately flash-frozen in liquid nitrogen, and ground into powder. DNA extraction was performed using the Universal DNA Kit (Mei5bio, China), following the manufacturer's protocol.

COI was amplified using the primer pair LCO1490 and HCO2198 (Folmer et al. 1994). For nuclear 18S rRNA gene amplification, three primer pairs were used sequentially: (1) F: AACCTGGTTGATCCTGCCAGT, R: CCAACTACGAGCTTTTTAACTG; (2) F: CGGTAATTCCAGCTCCAATAG, R: CAGACAAATCGCTCCACCAAC; (3) F: AAGGGCACCACCAGGAGTGGAG, R: TGATCCTTCCGCAGGTTCACCT (Apakupakul et al. 1999; Borda and Siddall 2004).

PCR conditions were as follows: initial denaturation at 94 °C for 2 min; 30 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 60 s; and a final extension at 72 °C for 10 min. PCR products were purified from 1% agarose gels and sequenced by Qingke Biotech (Beijing, China) using an ABI 3730XL DNA sequencer (Applied Biosystems, USA).

For complete mitochondrial genome sequencing, the entire body of an additional specimen (with gut contents removed) was used for high-quality DNA extraction. Genomic DNA was subjected to quality control, including concentration measurement by fluorescence quantification and assessment of integrity and purity using 1% agarose gel electrophoresis (150 V, 40 min). For library preparation, 500 ng of high-quality DNA was fragmented by ultrasonic shearing. The fragmented DNA was size-selected with magnetic beads to obtain fragments of ~200–400 bp, followed by end-repair, A-tailing, and adaptor ligation. The ligated products were amplified by PCR and purified with magnetic beads. PCR products were then denatured and circularized to generate single-stranded circular DNA libraries, and remaining linear DNA was digested. The final libraries were quantified to ensure appropriate concentration and quality, and subsequently sequenced using both second-generation sequencing on the Illumina NovaSeq platform and third-generation sequencing on the Nanopore PromethION platform.

Phylogenetic analyses

The phylogenetic position of the new troglobiotic leech within Salifidae was estimated using concatenated sequences of mitochondrial COI and nuclear 18S rRNA genes. The ingroup salifid and outgroup erpobdelliform taxa was selected based on previous studies (Nakano and Nguyen 2015; Nakano et al. 2018; Bolotov et al. 2023; Tang et al. 2025) (Table 1). The alignment of COI sequences was trivial because no indels were observed. The 18S sequences were aligned using MAFFT v. 7.520 L-INS-I (Katoh and Standley 2013). The final concatenated alignment was 3111 bp in length (COI: 1267 bp; 18S: 1844 bp).

Phylogenetic trees were reconstructed using maximum likelihood (ML) and Bayesian inference (BI). Based on the Bayesian information criterion using ModelFinder (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017) implemented in IQ-Tree v. 2.2.2.6 (Minh et al. 2020), the best-fit partition scheme and optimal models were identified as follows: the 1st position of COI, SYM + G; the 2nd position of COI, HKY + I; the 3rd position of COI, HKY + G; and 18S, K2P + I. The ML tree was inferred using IQ-Tree v. 2.2.2.6, with non-parametric bootstrapping (BS) conducted with 1000 replicates. The BI tree and Bayesian posterior probabilities (PP) were estimated using MrBayes v. 3.2.7a (Ronquist et al. 2012). Two independent runs with four Markov chains were conducted for 10 million generations, and the tree was sampled every 100 generations. Parameter estimates and convergence were checked using Tracer v. 1.7.1 (Rambaut et al. 2018), and then, the first 25001 trees were discarded as burn-in.

Mitochondrial genome assembly, annotation, and phylogenetic analysis

The complete mitochondrial genome was assembled using a hybrid approach combining second-generation Illumina and third-generation Nanopore sequencing technologies. Initially, Illumina reads were assembled de novo using MitoZ (Meng et al. 2019), and refined with GetOrganelle (Jin et al. 2020) to produce longer and more accurate scaffolds. These scaffolds were then used to filter Nanopore reads via Minimap2 (Li 2018), and the filtered long reads were assembled into a circular genome using Flye (Kolmogorov et al. 2019). To improve assembly accuracy, the MitoZ-based scaffold and the Flye-assembled genome were aligned, and any gaps in the Illumina assembly were filled with corresponding Nanopore sequences. The finalized mitochondrial genome was circular and complete.

Gene annotation was performed using the MITOS2 web server (Donath et al. 2019), which predicts protein-coding genes, tRNAs, and rRNAs based on reference invertebrate mitochondrial models. The circular genome was then visualized using OGDRAW (Greiner et al. 2019), and gene boundaries were manually curated to ensure accuracy.

Phylogenetic analysis of the complete mitochondrial genome was conducted using the Maximum Likelihood (ML) method. The procedure, including model selection, bootstrap analysis, and tree construction, followed the same parameters and methods described above for the mitochondrial COI and nuclear 18S rDNA analyses.

Data availability statement

The raw sequence data generated in this study have been deposited in public repositories. The COI and 18S rRNA gene sequences are available from the National Center for Biotechnology Information (NCBI) under accession numbers PV370489 and PV368445, respectively. The raw reads of the mitochondrial genome have been deposited in the China National Center for Bioinformation (CNCB) under BioProject accession number *Troglobdella guizhouensis* gen. et sp. nov. PRJCA043208.

Results

Taxonomy

Family Salifidae Johansson, 1910

***Troglobdella* Liu & Nakano, gen. nov.**

https://zoobank.org/527D339E-9C44-4068-BC0F-E24780EC5470

Type species. *Troglobdella guizhouensis* Liu & Nakano, sp. nov.

***Troglobdella guizhouensis* Liu & Nakano, sp. nov.**

https://zoobank.org/7A951DE7-3CC5-4CF4-8928-6C7B99833C0B

Etymology. The genus name *Troglobdella* is derived from the Ancient Greek "troglos" meaning "cave" and "bdella" meaning "leech" referring to the genus' trait to subterranean habitats. We suggest the Chinese formal name as "yǐn dòng zhì shǔ" (隐洞蛭属). The species name "*guizhouensis*" is based Guizhou Province in China, where the species was discovered, with the suffix "-ensis" indicating geographical origin. We suggest the Chinese formal name as "guì zhōu yǐn dòng zhì" (贵州隐洞蛭).

Material examined. ***Holotype*:** GZ20240501; body length 37.4 mm, maximum body width 4.5 mm, anterior sucker width 2.0 mm, posterior sucker width 4.9 mm. Collected from Chishui Cave, Yangjiao Group, Zhaoshan Village, Shanqiao Street, Caohai Town, Weining County, Guizhou Province, China (26°53'42"N, 104°19'59"E; elevation: 2334 m) on January 19, 2024 (Figs 1–3).

***Paratypes*:** Five specimens (GZ20240502-GZ20240506), same collection data as for the holotype. The holotype is preserved intact, and the dissection of one paratype (GZ20240506) is illustrated in Fig. 3.

Table 1. Samples used for phylogenetic analyses. Voucher information on the specimens is accompanied by the International Nucleotide Sequence Databases (INSD) accession numbers of the markers. Sequences marked with an asterisk (*) were obtained for the first time in the present study.

Species	Voucher	INSD#	
		18S	COI
Salifidae			
Troglobdella guizhouensis gen. et sp. nov.		PV368445*	PV370489*
Barbronia borealis Bolotov, Eliseeva & Kondakov, 2023	RMBH Hir_0405 (holotype)	OQ941865	OQ940656
Barbronia gwalagwalensis Westergren & Siddall, 2004		AY786462	AY786455
Barbronia weberi Blanchard, 1897		DQ235608	DQ235598
Linta be Westergren & Siddall, 2004		AY786466	AY786460
Mimobdella japonica Blanchard, 1897	KUZ Z179	AB663650	AB679658
Odontobdella blanchardi (Oka, 1910)	KUZ Z180	AB663651	AB938004
Odontobdella gaowangjiensis Yin & Liu, 2025	HNGWJ04 (18S); HNGWJ01 (COI)	PP532681	PP510626
Salifa motokawai Nakano & Nguyen, 2015	VNMN 2015.65 (holotype)	LC029434	LC029431
S. perspicax Blanchard, 1897	isolate 014	HQ336377	HQ336343
Shibabdella wulingensis Tang & Liu, 2025	JSUshiba01		PQ860767
Outgroup			
Erpobdella japonica Pawłowski, 1962	KUZ Z178	AB663648	AB679654
Gastrostomobdella ampunganensis Nakano, 2018	ZRC.ANN.0083 (holotype)	LC274517	LC274551
Gastrostomobdella monticola Moore, 1929	UNIMAS/A3/BH01/10	AB663649	AB679656
Orobdella whitmani Oka, 1895	KUZ Z45 (topotype)	AB663657	AB679668

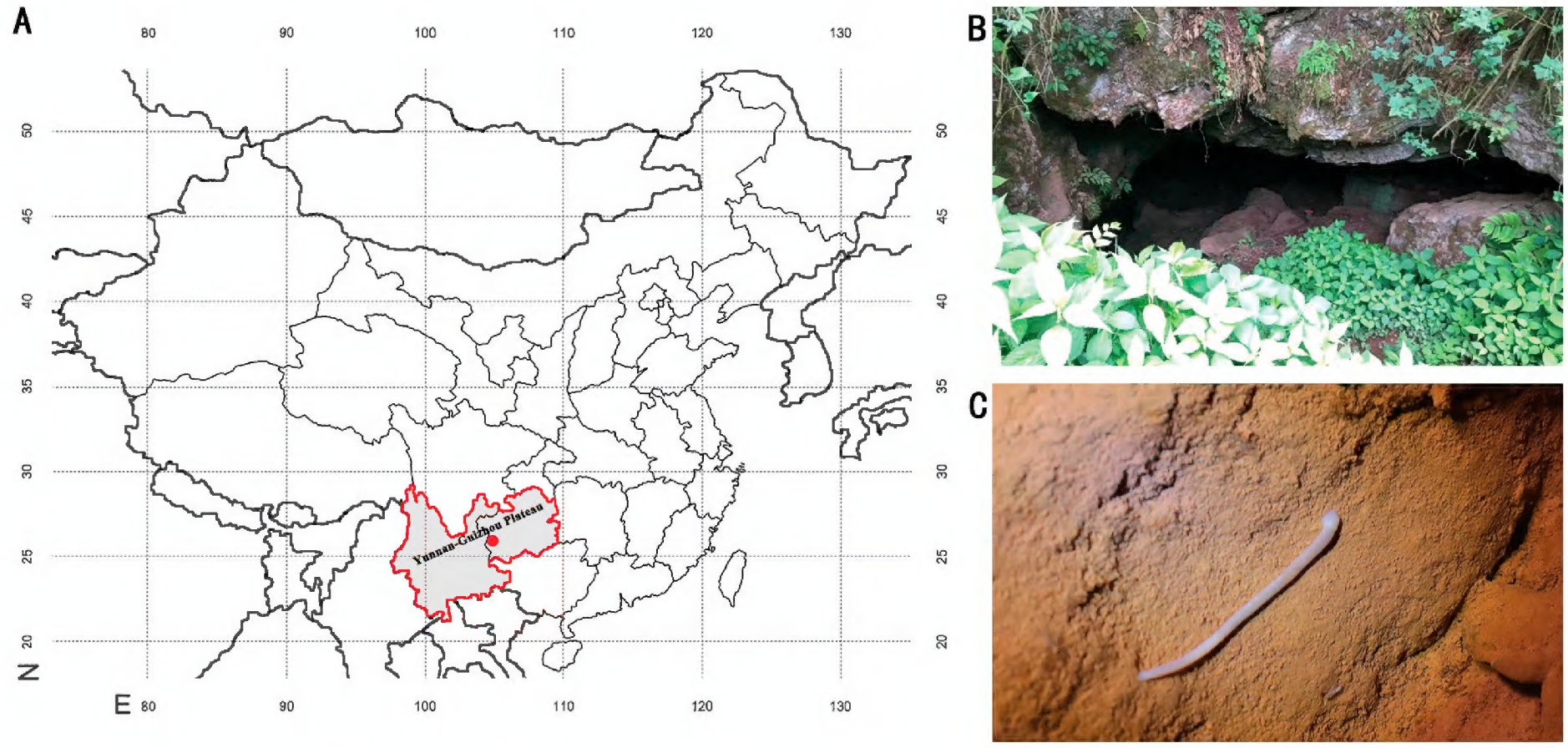

Figure 1. Collection site of *Troglobdella guizhouensis*. **A.** Map of geographic collecting location. The red line area indicates the Yunnan-Guizhou Plateau (Liu et al. 2025), and the red dot indicates the sample collection point; **B.** The entrance of the cave; **C.** A leech living in the puddle.

All type materials are deposited at the Engineering Research Center for Exploitation & Utilization of Leech Resources in Universities of Yunnan Province, Kunming University, Kunming, China.

Diagnosis. *Troglobdella guizhouensis* can be distinguished from the other salifid genera as well as other species by the following combination of characters: body milky white with no pigments; without eyes; oral sucker well developed, distinctly laterally expanded; mid-body somites six-annulate; gonopores separated by four annuli; testisacs multiple; pharynx strepsilaematous; lacking preatrial loop of male paired ducts; paired atrial cornua conical, curved laterad; atrium short, globular; ovisacs descending to anterior of somite XIV, then turned several times in each of posterior parts, forming globular mass.

Description. Aquatic predatory leech. Body firm and muscular, medium sized, gradually widening in caudal direction, length 38.1 ± 4.8 mm (n = 6), maximum body width 4.0 ± 0.3 mm, width of anterior sucker 2.3 ± 0.2 mm, width of caudal sucker 4.8 ± 0.5 mm. Caudal sucker diameter obviously wider than maximal body breadth (Fig. 2A, B).

Annulation of somites I–VII comprising 15 annuli altogether; 1st annulus completely merged with prostomium,

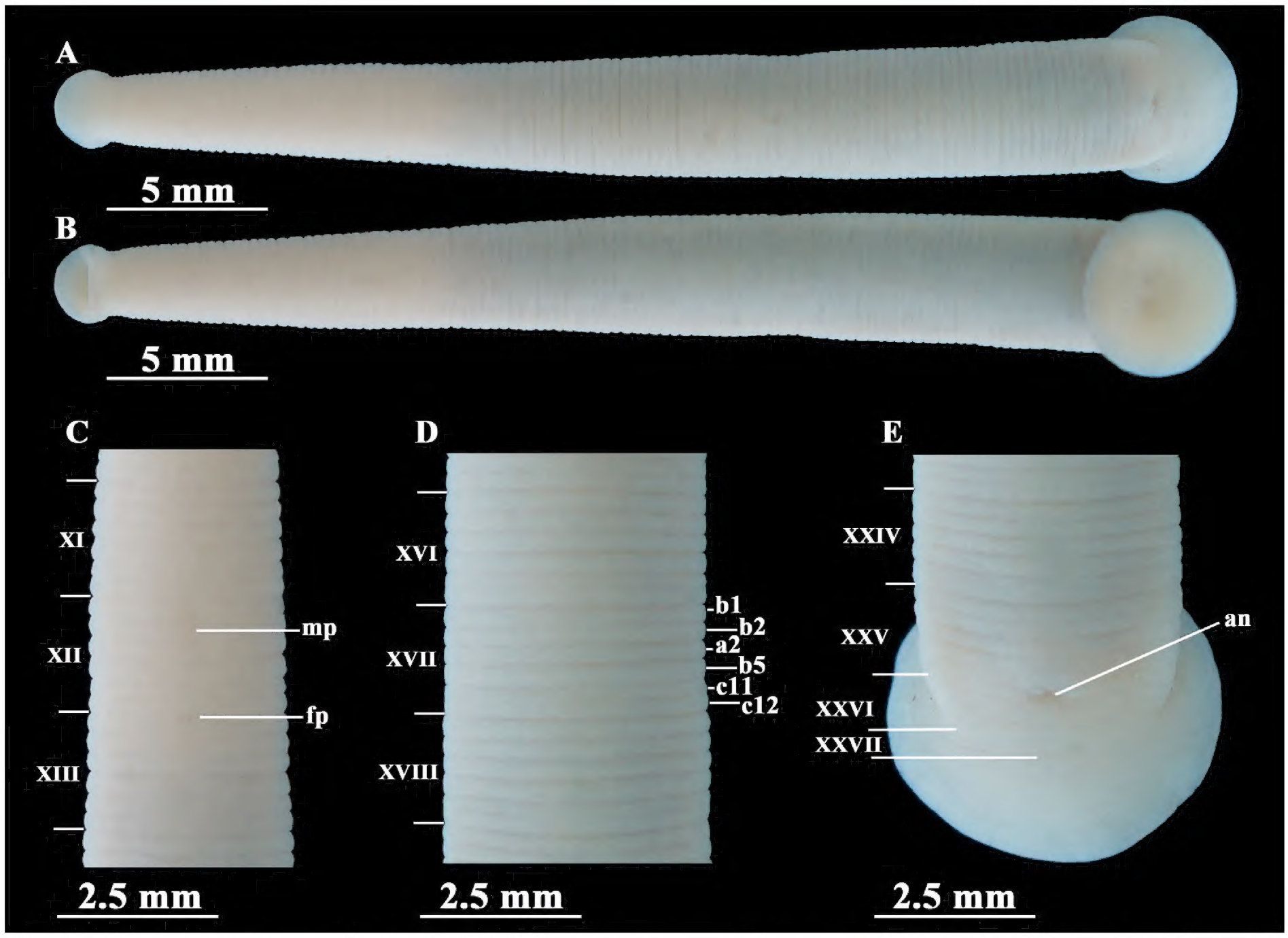

Figure 2. External morphology of the holotype of *Troglobdella guizhouensis*. **A.** Dorsal view of the entire body; **B.** Ventral view of the entire body; **C.** Ventral view of somites XI-XIII showing the positions of male and female gonopores; **D.** Dorsal view of somites XVI-XVIII showing segmentation pattern; **E.** Dorsal view of somites XXIV-XXVII. Abbreviations: fp, female gonopore; mp, male gonopore; an, anus.

then 2nd (peristomium) > 3rd–15th annuli; 6th and 7th annuli forming posterior margin of oral sucker. Somite VIII quinquannulate, a1 (with obvious secondary furrow, b1, b2) + a2 + b5 + c11 + c12. Somites IX–XXIV sexannulate (Fig. 2C–E), b1 + b2 = a2 = b5 = c11 = c12; in somites XI–XII, b5 of each somite generally with slight secondary furrow. Annulation of somites XXV–XXVII comprising more than 8 annuli altogether; 120th being last complete annulus on venter; anus between 120th and 121st annulus with more than 4 post-anal annuli. Accordingly, annulation of somites XXV–XXVII tentatively interpreted as follows (Fig. 2E): somite XXV quadrannulate, b1 + b2 + a2 + a3 (b5, b6), a3 being last complete annulus on venter; somite XXVI triannulate, a1 (b1, b2) + a2 + a3; somite XXVII uniannulate; anus at somite XXV/XXVI with more than 4 post-anal annuli. Somite X b5 and somite XIII a2, respectively, first and last annuli of clitellum.

Male gonopore in posterior margin of somite XII b2. Female gonopore in somite XII/XIII. Gonopores separated by four annuli (Fig. 2C).

Anterior ganglionic mass in 10th and 11th annuli (somite VI b5 and b6). Ganglion VII in 13th annulus (somite VII a2). Ganglia VIII and XII, of each somite, in a2 and b5. Ganglia IX, XII, XV, of each somite, in a2. Ganglia X, XIII, XIV, XVI-XX, of each somite, in b2 and a2. Ganglia XXI-XXIII, of each somite, in b2. Ganglion XXIV in b1 and b2. Ganglion XXV in somite XXIV c11. Ganglion XXVI in 117th annulus (somite XXV b1), coalescing with posterior ganglionic mass. Posterior ganglionic mass in 118th-121st annuli (somite XXV b2-somite XXVI a1).

Eyespots absent. Papillae numerous, minute, hardly visible, one row on every annulus, and two rows on annuli with secondary furrow.

Nephridiopores in 17 pairs, one each situated ventrally at posterior margin of a1 of somite VIII, and at posterior margin of b2 of each somite in IX-XXIV.

Pharynx strepsilaematous, reaching to somite XV b1, with 3 myognaths separated by triangular paragnaths, each myognath bearing two minute stylets arranged longitudinally in tandem, parallel to body axis. Crop tubular acecate, reaching to somite XIX b1; sphincter between crop and intestine undeveloped. Gastropore and gastroporal duct absent. Intestine tubular, thin-walled, acecate, reaching to XXIII b5. Rectum tubular, thin-walled, reaching straight to anus.

Testisacs multiple, uncountable, on each side in somite XVII b5 to XXIV b2. Paired sperm ducts coiled, narrowing at junction with atrial cornua, then running proximally toward atrial cornua; both sperm ducts in

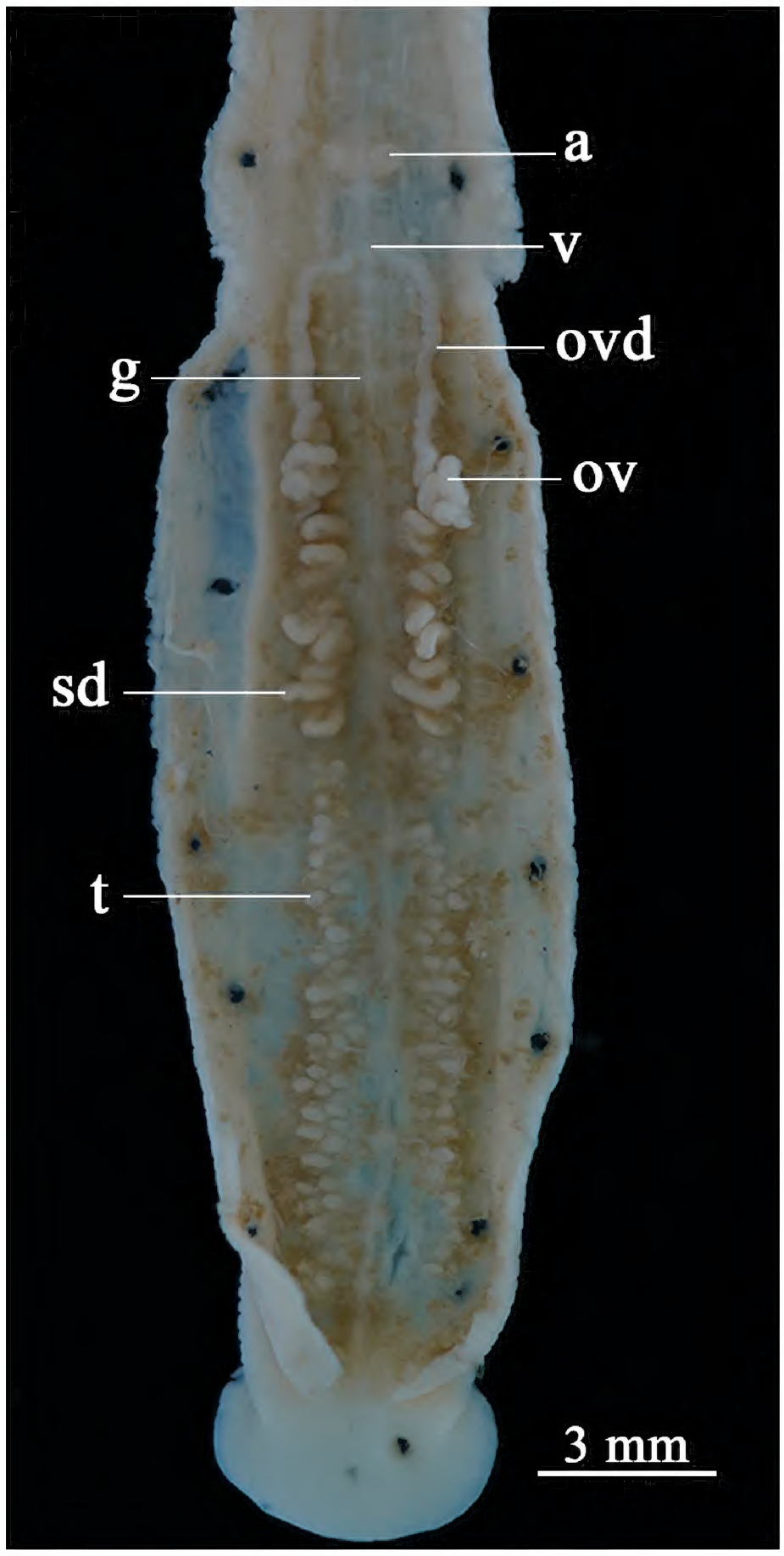

Figure 3. Dorsal view of reproductive system (paratype GZ20240506). Abbreviations: a, atrium; g, ganglion; ov, ovisacs; ovd, oviduct; sd, sperm duct; t, testisac; v, vagina.

somite XII b2 to somite XVII b5. Pair of atrial cornua conical, curved laterad, in XII b2 and a2 (Fig. 3). Atrium short, muscular, globular, in XII b2 and a2. Penis sheath and penis absent.

One pair of ovisacs, thin walled, slightly folded, tubular, descending to anterior of somite XIV, both ovisacs turned several times in each of posterior parts, forming globular mass (Fig. 3); in GZ20240504, right ovisac turned anteriorly in XIV a2, then reaching to XIV b2 and turned posteriorly, descending again to XIV a2, left ovisac turned anteriorly in XIV b2, then reaching to XIII c12 and turned posteriorly, descending again to XIV b2; both ovisacs, in XIII b2, turned proximally towards female gonopore, converging in anterior margin of XIII b1 and then directly descending to female gonopore.

Habitat. *Troglobdella guizhouensis* was found in shallow puddles approximately 30–50 meters from the entrance of Chishui Cave. The species likely feeds on aquatic invertebrates such as insect larvae and may burrow into sediment to avoid desiccation during dry periods or winter months.

Molecular phylogenetic position

The ML (Fig. 4) and BI (not shown) trees had almost identical topologies, and also generally agreed with those in the previous studies (Nakano and Nguyen 2015; Bolotov et al. 2023; Tang et al. 2025; Yin et al. 2025). The family Salifidae comprised three clade: 1) the Afro-Asian lineage (BS = 100%, PP = 1.0) consisting of *Salifa perspicax* Blanchard, 1897, *Salifa motokawai* Nakano & Nguyen, 2015, and *Linta be* Westergren & Siddall, 2004; 2) the *Barbronia* lineage (BS = 98%, PP = 1.0); and 3) East Asian lineage (BS = 63%, PP = 0.94) includes *Troglobdella guizhouensis*, *Shibabdella wulingensis*, *Odontobdella blanchardi* (Oka,1910), *Odontobdella gaowangjiensis*, and *Mimobdella japonica* Blanchard, 1897. The three Chinese species, *Troglobdella guizhouensis*, *Shibabdella wulingensis*, and *Odontobdella gaowangjiensis*, formed a lineage within the East Asian lineage, but this relationship was not supported (BS < 50%, PP < 0.5). Due to the low branch support, a sister relationship between a cave-dwelling salifid clade and *Odontobdella* cannot be ruled out, and therefore the possibility of multiple independent cave colonizations should be considered with caution. Nonetheless, *Troglobdella guizhouensis* represents a distinct lineage among the salifid taxa endemic to East Asia.

Mitochondrial genome assembly and annotation

The complete mitochondrial genome of *Troglobdella guizhouensis* was assembled into a circular molecule of 15,732 bp, containing 13 protein-coding genes (PCGs), 22 tRNA genes, and 2 rRNA genes (Fig. 5A). The PCGs include ATP6, ATP8, ND1-ND6, ND4L, COX1-COX3, and CYTB, consistent with mitochondrial genomes of other Erpobdelliformes (Xu and Nie 2016).

Gene order in *Troglobdella guizhouensis* is identical to that of *Barbronia weberi* (Blanchard, 1897) (from China) but differs from *Barbronia* cf. *gwalagwalensis* Westergren & Siddall, 2004 (from Australia), which possesses three putative heavy-strand replication origins (Fig. 5B).

Codon usage analysis revealed strong codon bias in PCGs. Leucine (UUA) and valine (GUA) showed the highest relative synonymous codon usage (RSCU) values of 3.51 and 2.55, respectively (Fig. 5C). Start codon AUG and tryptophan codon UGG showed no bias (RSCU = 1).

A neighbor-joining tree based on mitochondrial genome sequences confirmed the monophyly of

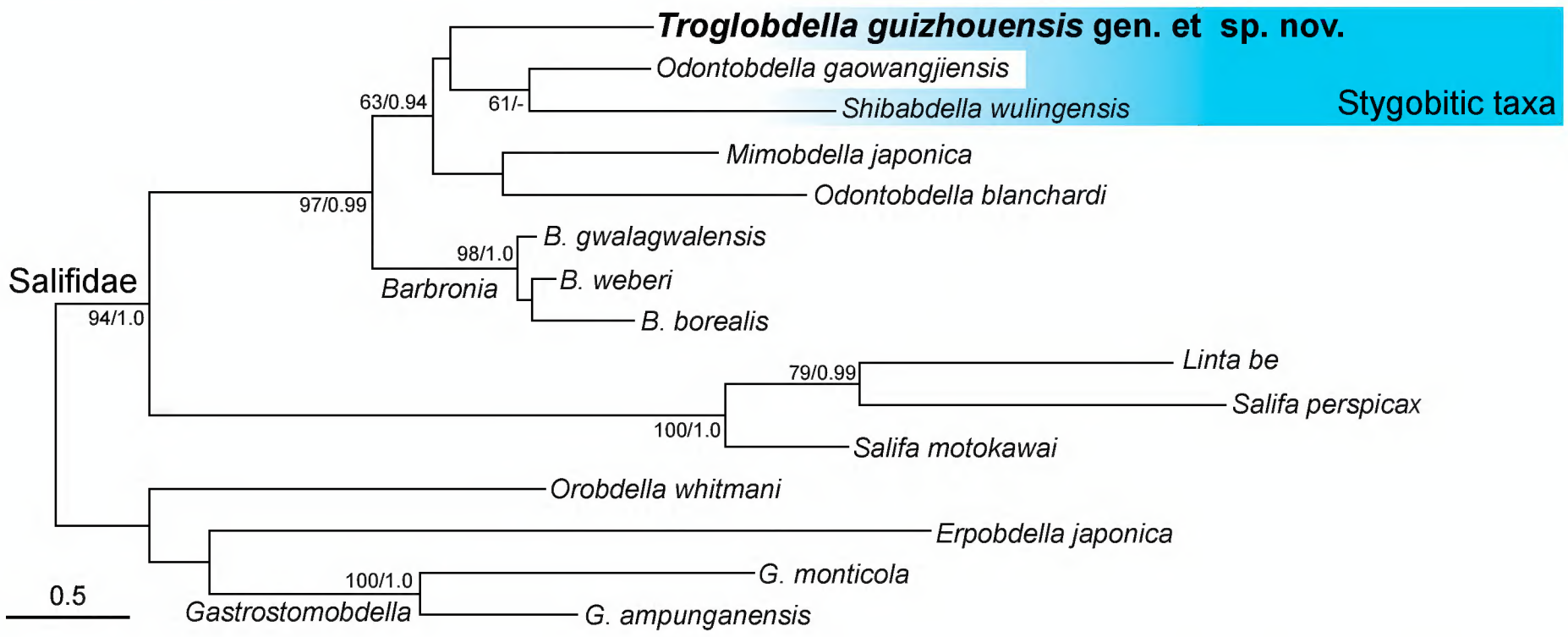

Figure 4. Maximum likelihood (ML) tree for 3111 bp of mitochondrial COI and nuclear 18S markers. Numbers on nodes indicate bootstrap values for ML ≥ 60% and Bayesian posterior probabilities ≥ 0.90. The scale bar represents the number of substitutions per site.

Erpobdelliformes within Arhynchobdellida, dividing them into two distinct clades: Salifidae and Erpobdellidae. *Troglobdella guizhouensis* formed a well-supported sister group with *Barbronia yanyuanensis*, *Barbronia weberi* and *Barbronia* cf. *gwalagwalensis*, corroborating its placement within Salifidae (Fig. 5D).

Discussion

The discovery of *Troglobdella guizhouensis* represents a significant addition to the diversity of cave-dwelling leeches and provides important insights into the evolution, taxonomy, and mitochondrial genomics of Salifidae. This newly described species not only expands the known ecological range of the family but also highlights the evolutionary plasticity of salifid leeches in adapting to extreme subterranean environments.

The morphological features of *Troglobdella guizhouensis*, including complete loss of pigmentation, absence of eyes, and a milky white body color, are characteristic of obligate cave-dwelling organisms (troglobionts) and are commonly observed in other cave-adapted invertebrates such as beetles, crustaceans, and planarians (Culver and Pipan 2009). These regressive traits are considered convergent traits to the aphotic, nutrient-limited cave environment, where selection favors energy conservation and enhanced non-visual sensory mechanisms (Poulson and White 1969).

Cave-related traits are rare among leeches. Prior to this study, only a few troglobiotic species had been reported, including two land leech species *Sinospelaeobdella cavatuses* Yang, Mo & Wang, 2009 (Yang et al. 2009) and *Sinospelaeobdella wulingensis* Liu, Huang & Liu, 2019 (Huang et al. 2019). Our findings support the hypothesis that cave colonization in Hirudinea may have occurred multiple times independently, particularly in the karst regions of southern China, where extensive subterranean systems offer ecological isolation and promote lineage diversification (Liu 2021).

Morphologically, *Troglobdella guizhouensis* can be distinguished from all other known salifid genera by a unique combination of annulation pattern, reproductive anatomy, and absence of a preatrial loop. These features justify the establishment of a new genus under standard taxonomic practice.

Phylogenetic analyses based on mitochondrial COI and nuclear 18S rRNA markers indicate that the five East Asian salifid species do not cluster into a single clade, but instead form two distinct lineages. *Troglobdella guizhouensis*, *Odontobdella gaowangjiensis*, and *Shibabdella wulingensis* group together in one clade, whereas *Mimobdella japonica* and *Odontobdella blanchardi* form another. This topology suggests that the diversification of East Asian salifids is more complex than previously recognized and may involve multiple independent evolutionary events (Nakano and Nguyen 2015; Bolotov et al. 2023).

Our phylogenetic analyses recovered *Troglobdella guizhouensis* as a distinct lineage within the East Asian salifids. However, because of the relatively low branch support within this clade, we cannot rule out the possibility of a sister relationship between the cave-dwelling salifids and *Odontobdella*. Notably, the separation of *Odontobdella gaowangjiensis* from *Odontobdella blanchardi* raises questions about whether *Odontobdella gaowangjiensis* should be retained within genus *Odontobdella* (Yin et al. 2025). Therefore, the hypothesis of multiple independent cave colonizations in salifids should be considered with caution.

The complete mitochondrial genome of *Troglobdella guizhouensis* (15,732 bp) falls within the size range observed in other Erpobdelliformes, with conserved gene content and codon usage. Notably, the gene order in *Troglobdella guizhouensis* is identical to that of *Barbronia weberi*, suggesting strong conservation within this lineage. However, differences in replication

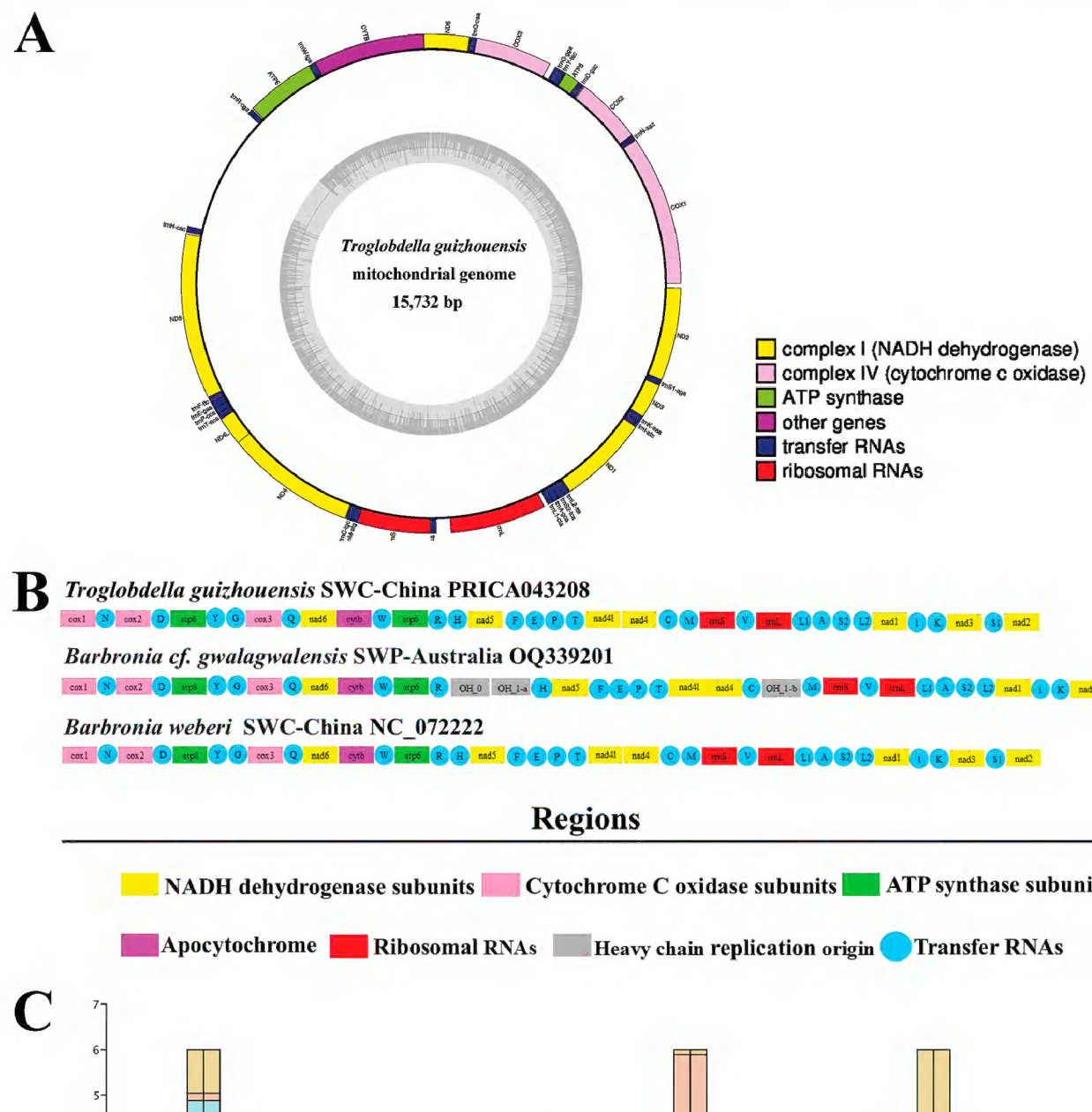

Figure 5. Mitochondrial genome assembly and annotation of *Troglobdella guizhouensis*. **A.** Circular genome map of mitochondrial DNA; **B.** Mitochondrial gene order of *Troglobdella guizhouensis* and closely related congeneric species; **C.** Codon usage bias analysis of the mitochondrial genome; **D.** Phylogenetic analysis of *Troglobdella guizhouensis* and related species. The scale bar represents the number of substitutions per site.

origin structure between *Troglobdella guizhouensis* and *Barbronia gwalagwalensis* (from Australia) reflect lineage-specific rearrangements, possibly associated with long-term geographic isolation. Codon usage analysis of Troglobdella guizhouensis revealed strong codon bias in PCGs, with leucine (UUA) and valine (GUA) exhibiting the highest RSCU values, while start codon AUG and tryptophan codon UGG showed no bias. In metazoan mitochondria, every known genetic code change is conserved within its respective phylum (Abascal et al. 2006).

Whether the observed codon usage bias is associated with translation efficiency under resource-limited conditions requires further investigation.

The Yunnan-Guizhou Plateau, one of the four major plateaus in China, is a hotspot for biodiversity (Wang et al. 2022), yet its leech fauna remains poorly documented. The discovery of *Troglobdella guizhouensis* highlights the importance of continued biospeleological surveys in karst regions of Southwest China, where ecological isolation fosters high rates of endemism. Remarkably, this species was collected at an altitude exceeding 2,300 meters, which is likely a world record for cave-dwelling leeches and underscores the extreme environmental conditions under which it survives. Given the vulnerability of cave ecosystems to human disturbance, including tourism, pollution, and hydrological alteration, *Troglobdella guizhouensis* may be highly sensitive to environmental change. Its limited distribution and specialized habitat warrant conservation attention and potentially future IUCN assessment.

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